Neural networks: Structure and repair
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Progress report
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Part 1:

Ground squirrel visual system

Progress on experiments:

1) To characterize the dendritic arborization of ganglion cells projecting to the superior colliculus (SC):

Nancy Rivera, a graduate student, is actively involved in this part of the project. She had been attempting to fill the dendritic tree of ganglion cells projecting to the SC with intracellular injections of fluorescent dyes (Lucifer Yellow, DiI). To identify these ganglion cells she injected rhodamine (red) or fluorescein (green) filled latex microspheres ("beads") into SC. These beads are transported retrogradely to cell bodies of ganglion cells projecting to the injected structure.

A second approach is also under way. Some excellent results have been obtained by injecting cholera toxin B subunit (CTB) into the SC for retrograde tracing of ganglion cells. The CTB is then immunocytochemically detected. When working properly this procedure produces a Golgi-like image. Several morphological types have been identified and we are involved in a detailed analysis of differences in their dendritic arborization and retinal distribution.

2) To determine whether retinal ganglion cells project to more than one retinal projection target:

Red beads were injected into the left midbrain and thalamus and green beads into corresponding areas in the right hemisphere of the same animal. Labeled retinal ganglion cells in the temporal retinas project ipsilaterally and most labeled cells in the nasal portion have contralateral projections. Nevertheless, there is a portion of the nasal retina where ipsilaterally and contralaterally projecting cells overlap. Very few clearly doubly labeled cells have been identified.

3) To study where there is a difference in the fine structural pattern of synaptic connections among different ganglion cell types:

We have identified presumed dopaminergic amacrine cells with an antibody against tyrosine hydroxylase (TH). Stained retinas were embedded and sectioned for observation with a Philips electron microscope. EM results indicate that TH-labeled amacrines make synaptic contacts with unlabeled amacrines, although the possibility that they may contact ganglion cells can not be ruled out.

4) To explore the variety of neuroactive substances in the ground squirrel retina:

TH was used to study the distribution of dopamine in the ground squirrel retina. TH-like immunoreactivity was exhibited by amacrine and interplexiform-like cells in the innermost portion of the inner nuclear layer (INL) and by displaced amacrines in the ganglion cell layer (GCL). Processes from the three types extended into sublamina 1 of the INL. A distance to the nearest neighbor analysis suggests the labeled amacrines in the INL are distributed in a non-random fashion. The mean overall density of labeled amacrines is 15 cells/mm^2.

Wilfredo López, another student, is actively involved in this part of the project. He is using immunohistochemical methods to investigate the location of somatostatin and substance P in the ground squirrel retina. Although he has been able to identify immunoreactive processes, he has had difficulty in labeling cell bodies. He is presently investigating the use of colchicine to interrupt axonal transport and thus concentrate the peptides in the cell's soma and, incorporating picric acid in his fixatives to enhance labeling.

5) Other experiments:

a) Experiments to determine the approximate percentage of displaced amacrine cells in the ganglion cell layer of the ground squirrel retina:

We compared the number of labeled cells in the GCL after HRP injections of optic tracts and target nuclei with the total number of neurons in this layer. We conclude that approximately one half of the neurons in the GCL are displaced amacrine cells, the other half are ganglion cells. The displaced amacrines are on the average smaller than the ganglion cells.

Part 2:

Formation, Maintenance and Plasticity of Synaptic Connections

An aim of the experiments in the project supported by the ONR is to determine whether synapse formation by neurons is specific to certain targets and to specific sites on these targets. For example do motoneurons specifically reinnervate muscle fibers at their

original synaptic sites vs their extrasynaptic regions. Cultures of adult motoneurons have been established. As a next step, conditions have been improved for isolating and maintaining intact adult muscle fibers in culture. These muscle fibers now survive and contract when electrically stimulated for more than 4 weeks *in vitro*. To determine whether motoneurons selectively reinnervate muscle fibers at their original synaptic sites it is essential to be able to localize these sites *in vitro*. The synaptic acetylcholine receptors have been localized using the acetylcholine receptor specific binding of fluorescently labeled alpha bungarotoxin. We have found that the synaptic sites can be localized even after the muscle fibers have been in culture for 4 weeks. This is a novel finding since for most dissociated muscle fibers the original synaptic acetylcholine receptors disappear within 6 days *in vitro*. We also find that, even at the light microscopic level, under phase optics, the original synaptic sites can be recognized from morphological criteria in the absence of the alpha-bungarotoxin labelling. The reliability of recognizing these sites is greater than 80%. This finding is important because it will allow original synaptic sites to be recognized in the absence of synapse labeling techniques and will facilitate the correlation of synapses formed on muscle fibers with original synaptic sites without the cumbersome need for staining the synaptic sites. Muscle fibers, maintained in culture for up to 4 weeks, have been fixed and embedded for electron microscopy to determine the morphological correlates of the original postsynaptic sites in these long-term cultured muscle fibers that correspond to the sites of bungarotoxin labeling.

Adult motoneurons extend limited processes when cultured on tissue culture plastic and require a specific frog laminin-rich extracellular matrix extract on which to be cultured. Experiments are underway to isolate this extract which will improve process outgrowth. However, in the absence of this extract, and in spite of the limited process outgrowth, motoneurons are been co-cultured with muscle fibers to determine whether they influence motoneuron process outgrowth. Initial results indicate that the muscle fibers release a neurotrophic factor that promotes more process outgrowth from the motoneurons than is seen for control neurons.

In conclusion, tissue culture conditions have been improved to allow experiments to test whether adult motoneurons form synapses specificity at the original synaptic or extrasynaptic sites on intact adult muscle fibers. The resent results set the stage for experiments to determine the differences between adult frog muscle fibers and those studied by other researchers that cause them in retain rather than lose their original synaptic site receptors *in situ* following enzyme dissociation. In addition, other experiments will eventually attempt to characterize the neurotrophic factor released by the muscle fibers.

PUBLICATIONS

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